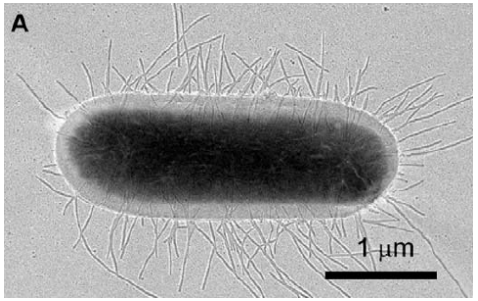


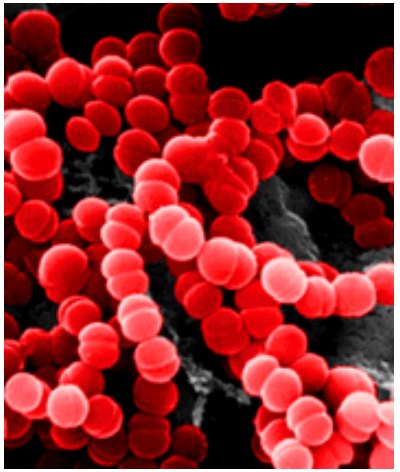
Structure and assembly of bacterial pili, from analysis of virulence-related gene clusters

Ted Baker

University of Auckland

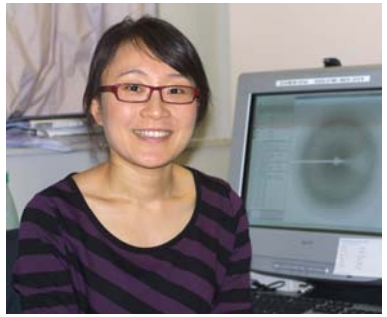
New Zealand

Context: virulence-associated proteins from *Streptococcus pyogenes*



- Serious human pathogen
  mild skin and throat infections
  serious invasive disease
    - septicemia, toxic shock, necrotising fasciitis
- Infection and disease mediated by:
    - secreted proteins (eg. superantigens)
    - cell-surface proteins (eg. adhesins)
- Focus here on cell-surface proteins
- Problem: Can often recognise them via attachment motifs
  - but most are of unknown function
  - significant host immune pressure
    ⟶ wide sequence variation

Mini-SG project on a virulence-associated gene cluster from *S. pyogenes*



M1

- Recognised as a pathogenicity island (~12 genes)
- Genes for two sortases – SrtB and Spy0129

Other genes

- Spy0125, Spy0128, Spy0130 have sortase cleavage motifs

Spy0125 (*cpa*, collagen binding)	VPPTG	**Sortase substrates**
Spy0128 (unknown function)	EVPTG	
Spy0130 (unknown function)	LPxTG	

PNAS

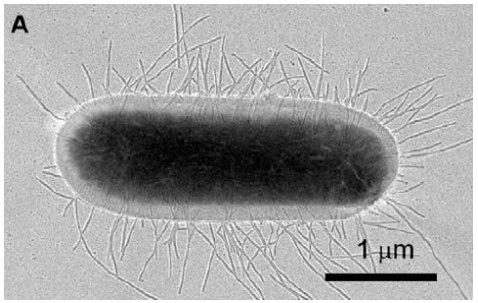
Group A *Streptococcus* produce pilus-like structures containing protective antigens and Lancefield T antigens

Marirosa Mora*†, Giuliano Bensi*†, Sabrina Capo*, Fabiana Falugi*, Chiara Zingaretti*, Andrea G. O. Manetti*, Tiziana Maggi*, Anna Rita Taddei‡, Guido Grandi*, and John L. Telford*§

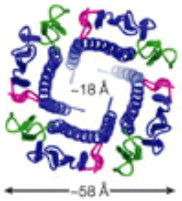
*Chiron Vaccines, Via Fiorentina 1, 53100 Siena, Italy; and ‡Centro Interdipartimentale di Microscopia Elettronica, University of Tuscia, 01100 Viterbo, Italy

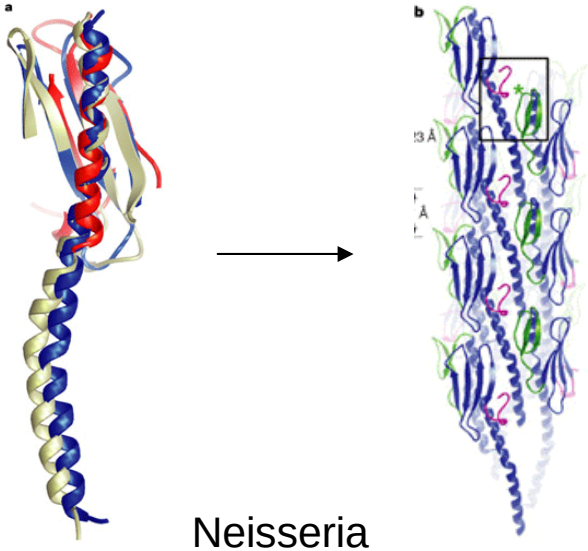
Communicated by Rino Rappuoli, Chiron Corporation, Siena, Italy, September 8, 2005 (received for review July 29, 2005)

Bacterial pili



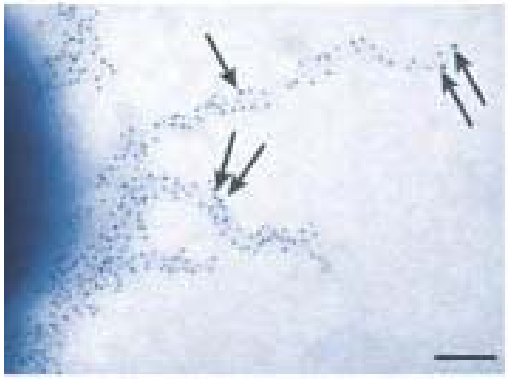
- Long polymeric hair-like structures
  - typically 100-500 copies of pilin subunits assembled into filaments
- Used for – adhesion to host cells
  - colonisation, biofilm formation etc
- Highly immunogenic (used for vaccines)
- Required to be physically very strong and stable
- Best known: Type 1 and Type IV pili from Gram-negative bacteria
  - pilins form rod-like bundles
  - non-covalently assembled



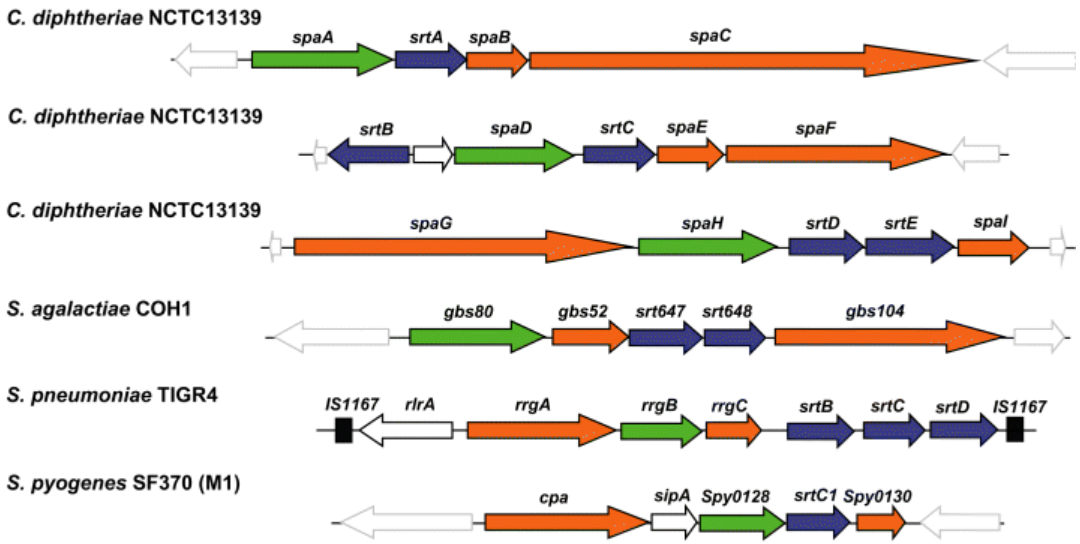


Neisseria

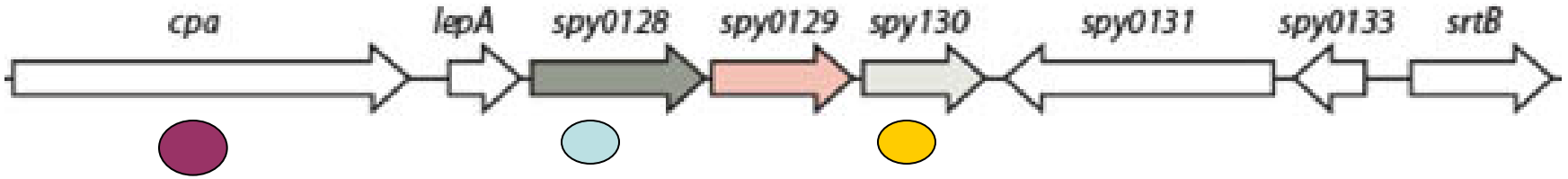
Pili in Gram-positive organisms



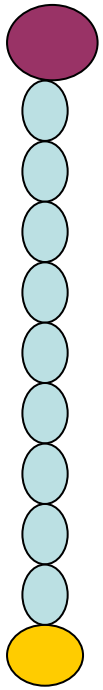
- Only discovered 5-10 years ago!
  - very long (1-2 μ), very thin (20-30 Å)
  - only visualised by EM with the aid of gold-labelled antibodies
- Discovered as genome sequences became available and predicted surface proteins could be expressed.
- Components encoded in small gene clusters together with one or more sortase genes



Pilus components for *S. pyogenes*

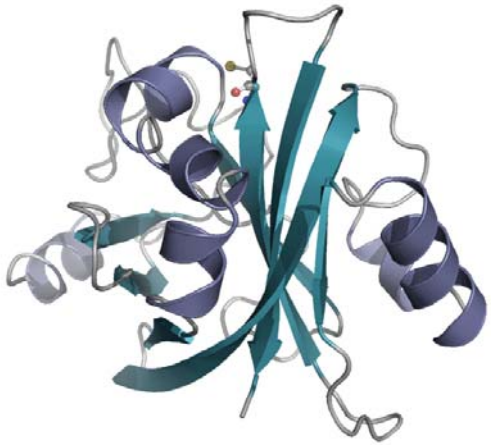


- Encoded in pathogenicity island, roles discovered by gene deletions:
- Sortase enzyme Spy0129 required for assembly
- LepA/SipA also required – unknown role
- Spy0128 is major pilus subunit ⟶ backbone
- Spy0125 and Spy0130 are ancillary pilin subunits
  - Spy0130 at the base – cell wall anchor
  - Spy0125 at the tip – adhesin that binds to cells
- Also: Spy0128 known to be highly immunogenic and extremely stable and protease-resistant
  - Lancefield T-antigen



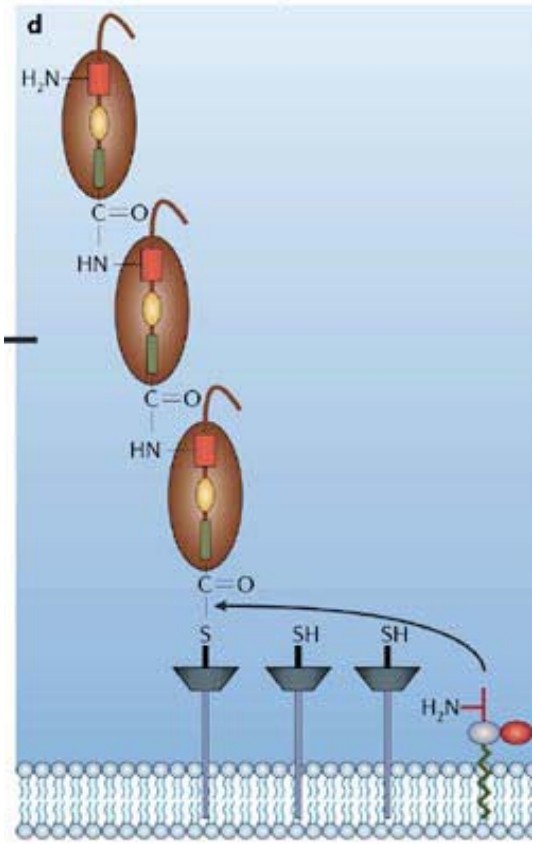
Sortase – Spy0129

- Sortases are cysteine transpeptidase enzymes
- Main activity – anchor proteins to the cell wall
  - catalyse formation of covalent isopeptide bond linking C-terminus to NH_2 group of peptidoglycan
- Subset of sortases assemble pili
  - join C-terminus subunit to NH_2 group of a Lys residue on next
    ⟶ covalent polymer



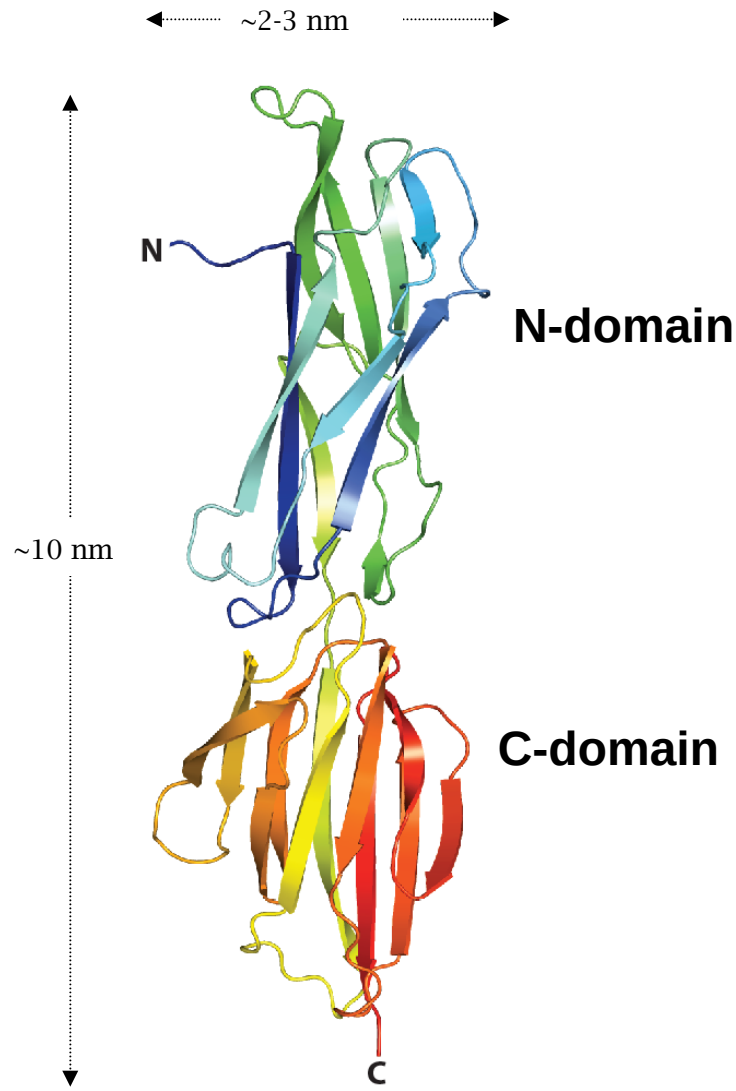
Spy0129

HaeJoo Kang

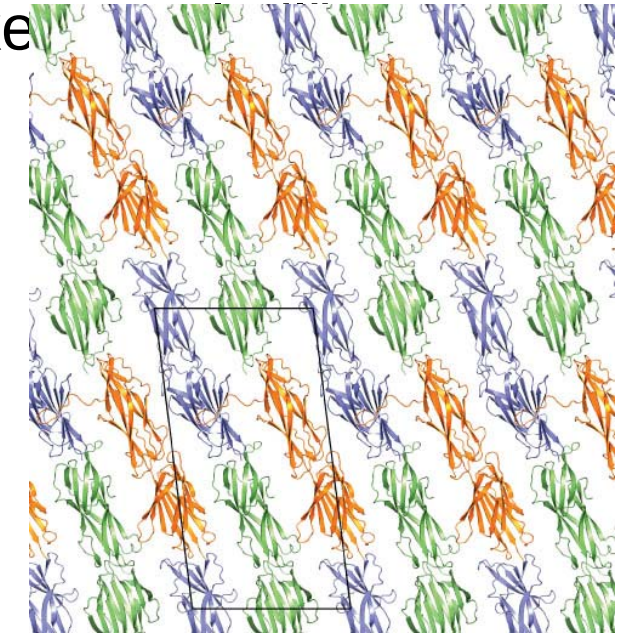


Structural biology of Gram-positive pili

Structure of the pilin subunit Spy0128

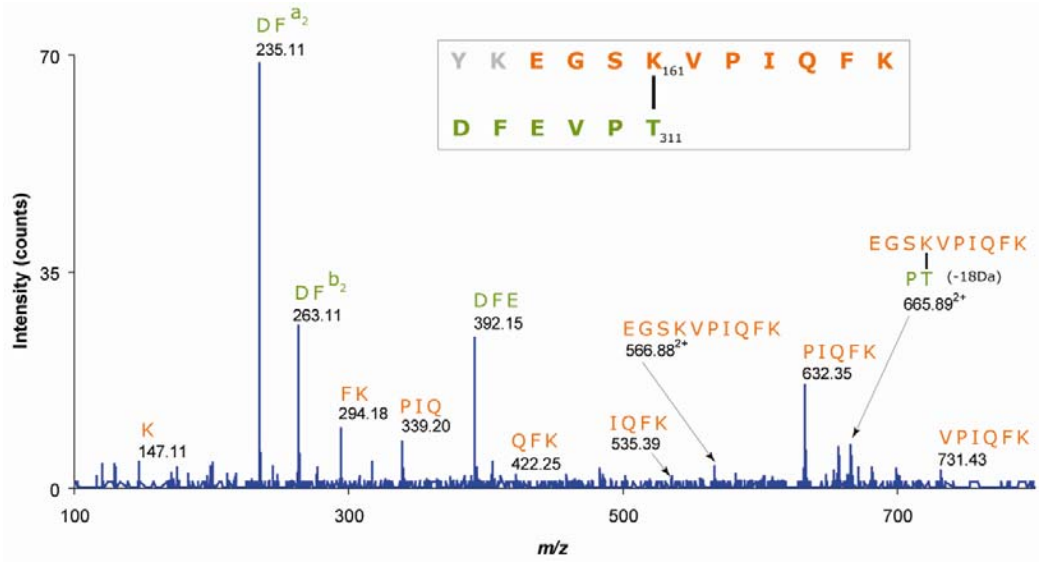


- Structure solved at 2.2 Å resolution
- Elongated molecule folded into two Ig-like domains
- Forms pilus backbone
- Powerful insight from crystal packing
  - molecules stacked end-on-end (like

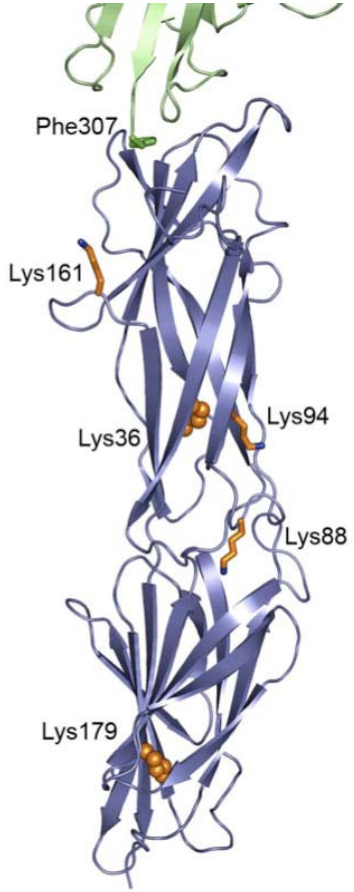


Discovery of Lys involved in polymerisation

- Only one Lys is conserved in all strains and is in position to join to C-terminus of next subunit and generate extended pilus – Lys161 (if our crystal packing model was correct!)
- Proved by mass spectrometry of native pili



HaeJoo Kang
Fiona Clow
Martin Middleditch



And now, for a complete surprise

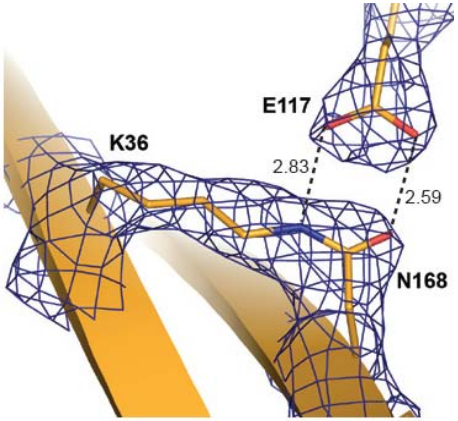
There are known knowns. These are the things that we know that we know. There are known unknowns. That is to say, there are things that we know we don't know. But there are also unknown unknowns. These are things we don't know we don't know.

Rumsfeld, D. (2002). *Press briefing*.

Unexpected density

Kang et al. (2007)
Science 318
1625-1628

- Discovered continuous electron density joining Lys and Asn side chains

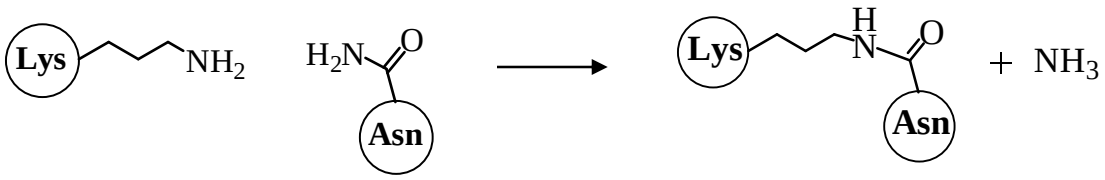


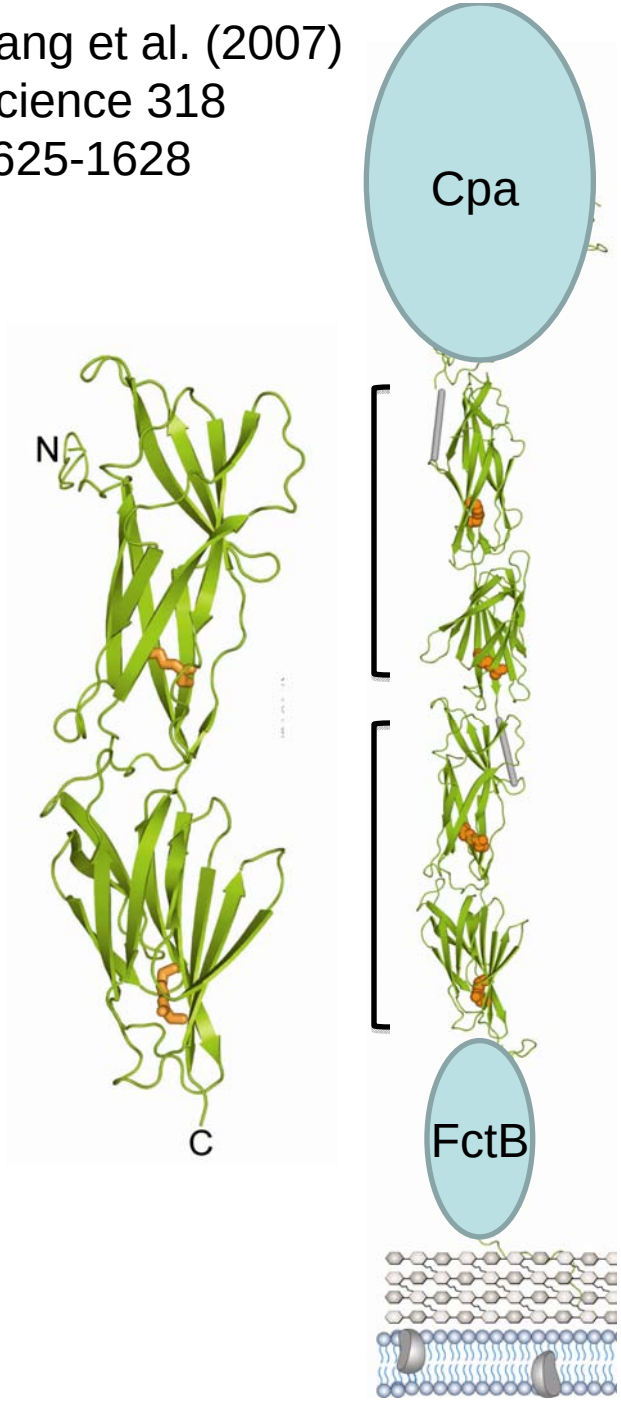
Isopeptide bonds

Spontaneously formed on folding

One in each domain

Catalytically-essential Glu residue





- Verified by mass spectrometry
- Accounts for great stability of these pili
  - covalent linkages between subunits
  - covalent internal cross-links along shaft

What about the other pilus subunits?

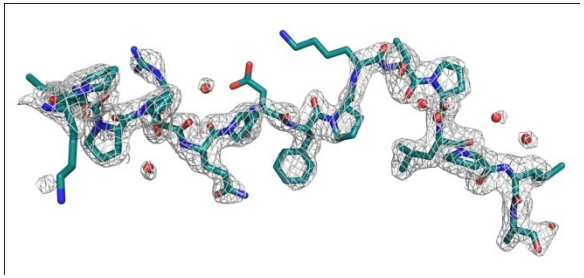
Basal subunit

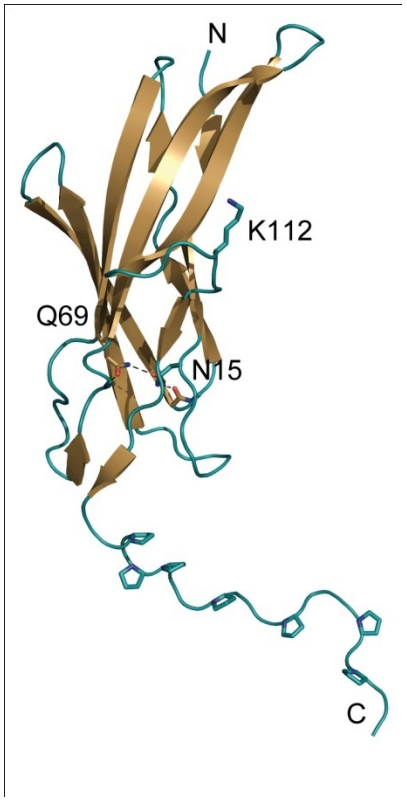
Adhesin at the tip

Basal pilin subunit FctB

Christian Linke
Paul Young
JBC (2010) 285, 20381-20389

- Structure solved at 1.9 Å
  - same fold as N-terminal domain of Spy0128 and equivalent Lys for incorporation into pilus
- Explains why same sortase can link FctB to the shaft



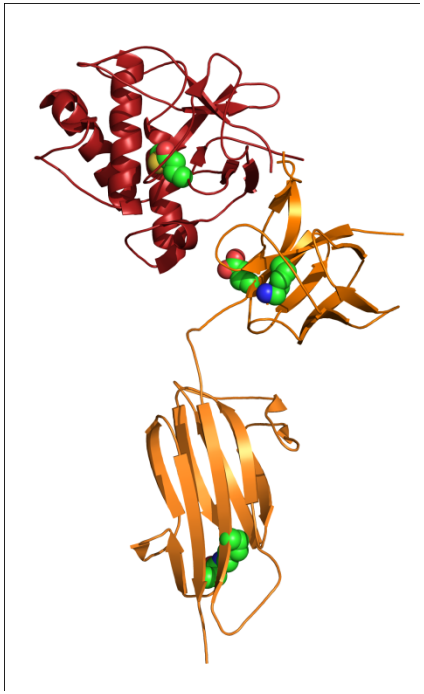


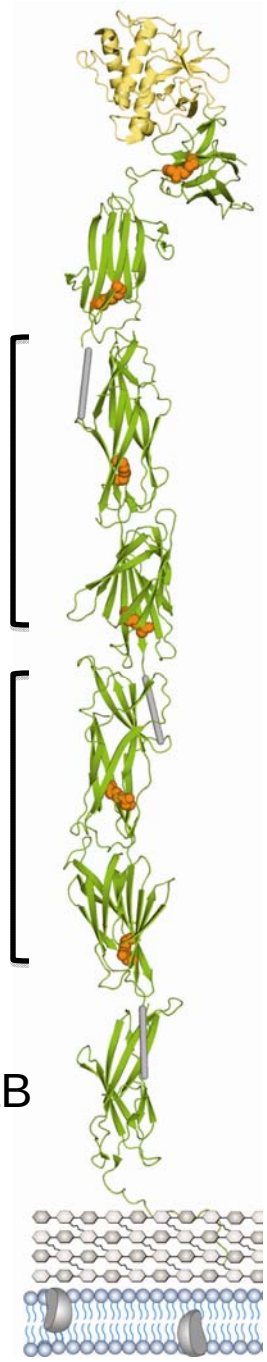
- Proline-rich tail at C-terminus
  collagen-like helix
  reaches into cell membrane?
- Attached to membrane by different sortase

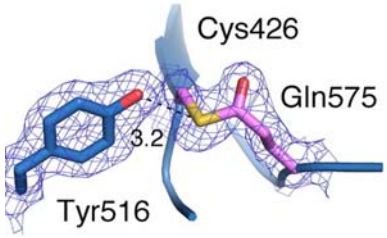
Adhesin subunit Cpa

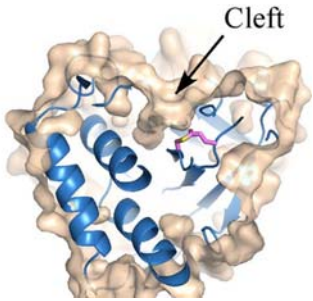
- Structure solved at 2.1 Å resolution
- Three domains
  - domain 1 has novel α/β fold and is essential for adhesion
- Isopeptide bonds in domains 2 and 3
- Domain 1 has unique thioester bond between Cys and Gln side chains
- Similar to Complement
  Covalent binding to host cells?

Pointon et al (2010) JBC 285 33858-33866



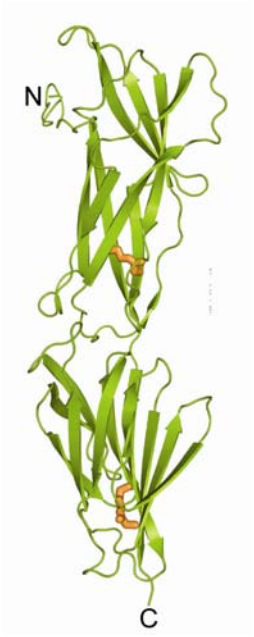




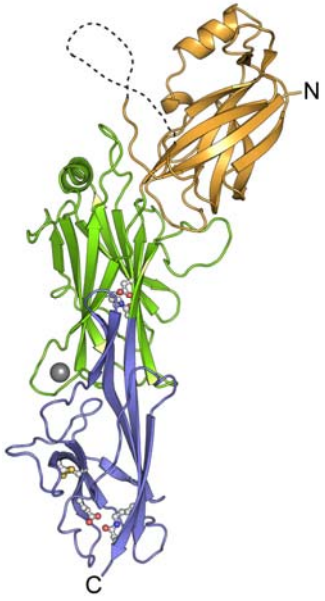


Other Gram-positive pili

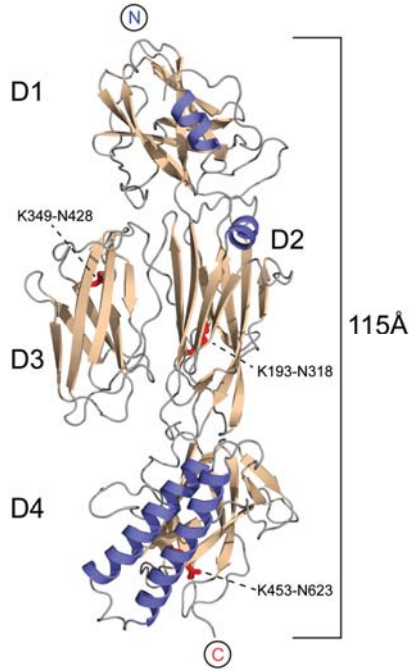
- Sizes of pilin subunits vary widely
  - and very little sequence identity (isopeptide bonds not easily identified by sequence analysis)
- Found in crystal structures of major pilin subunits



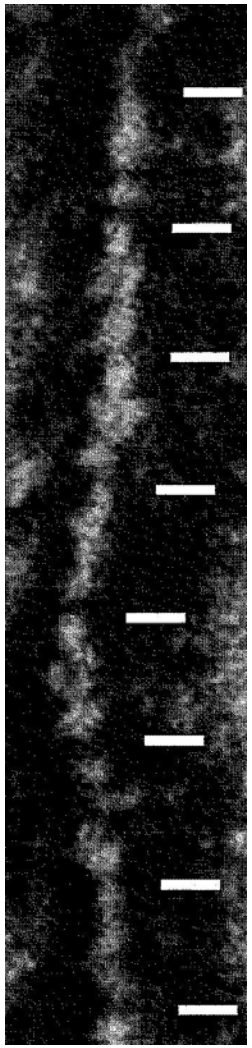
S. pyogenes Spy0128
2 domains



C. diphtheriae SpaA
3 domains



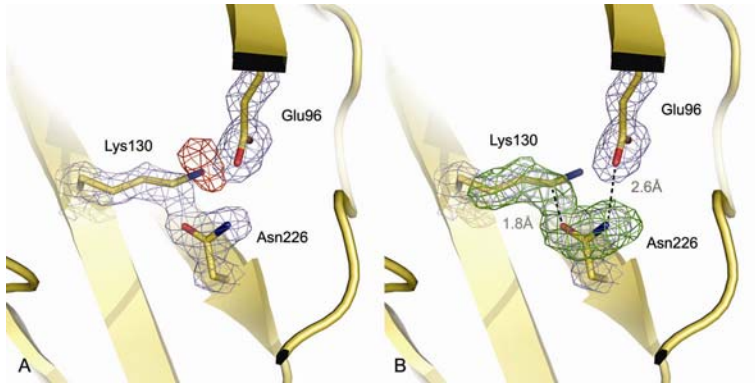
S. pneumoniae RrgB
4 domains



Are isopeptide bonds present in other proteins?

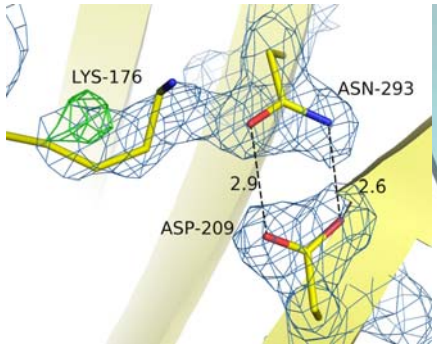
From structural searches

- 3D template search of PDB (Jess)
- Search for Lys-Asn-Glu/Asp triads
- Found several previously unrecognized isopeptide bonds in cell matrix-binding surface proteins of Gram-positive bacteria

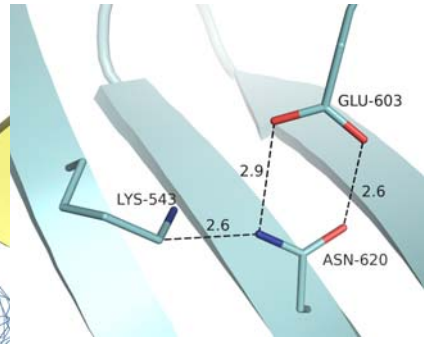


Minor pilin from S. agalactiae

- Collagen-binding protein
  Cna from *S. aureus*
  - Collagen binding Cna A domain
  - Structural Cna B domain



Cna A



Cna B

Evidence for a very widespread family

- Bacterial pili
- Cell surface adhesins

Either

- covalently linked pilin subunits

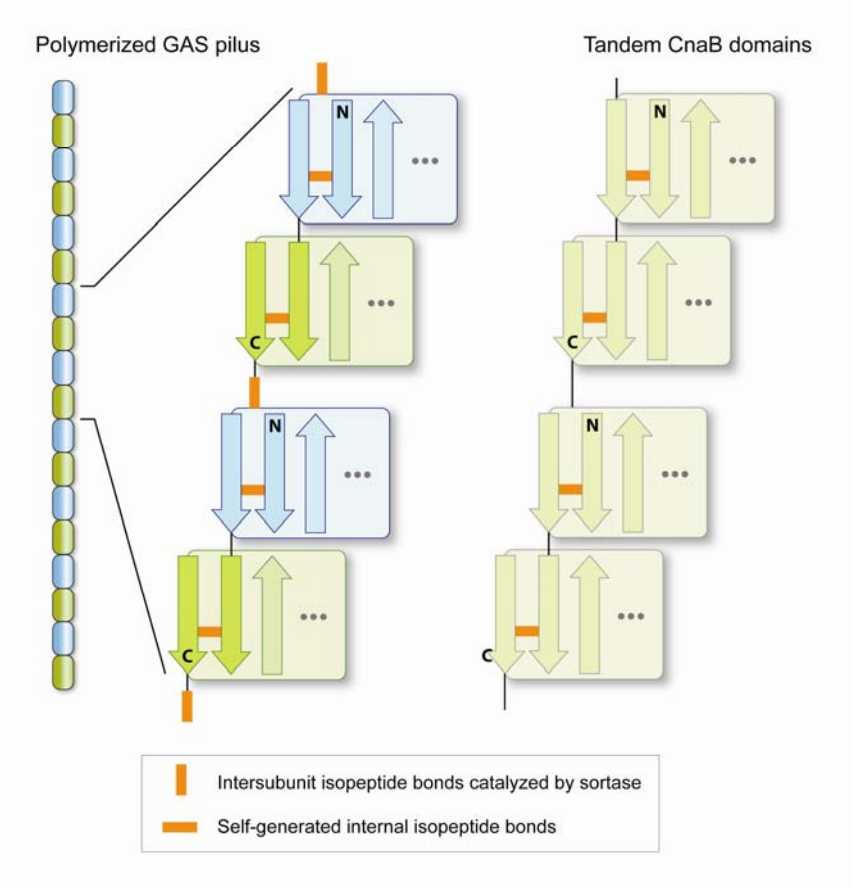
(*S. pyogenes*, *S. pneumoniae*, *S. agalactiae*, *B. anthracis*….)

Or

- tandem domains of multi-domain proteins

(from *S. aureus*, *S. suis*, *S. gordonii*, *S. pyogenes*…)

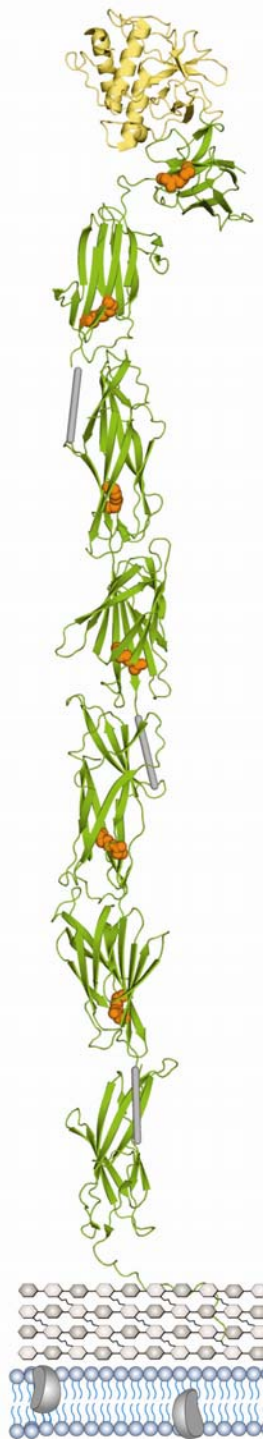
- Built of similar Ig-like domains
- And isopeptide bonds to give stability and mechanical strength



Kang & Baker (2011) *Trends in Biochem. Sci.* 36, 229-237.

Conclusions

- Illustrates ability of structural analysis to reveal novel, unexpected features of protein structure
  - intramolecular isopeptide cross-links
- Pilus: have structural model for complete assembly
  - and understand its strength and stability
- Structures ⟶ basis for therapeutic drug design
  - could block the adhesin at the tip
  - could inhibit sortase (stop assembly and attachment)
- Potential role in vaccine development
  - pili are highly immunogenic
- Unanswered questions:
  - how does assembly take place at cell surface?
  - what are targets on host cells?
  - what is the mechanism of adhesion?



Acknowledgments

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- Christian Linke
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- Paul Young
- Thomas Proft

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- Marsden Fund of NZ
- Maurice Wilkins Centre

That’s all Folks!

MAURICE WILKINS CENTRE
FOR MOLECULAR BIODISCOVERY